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13. ABSTRACT *(Maximum 200 Words)*

Our working hypothesis is that induced p120 loss in the breast will impair E-cadherin function leading to (1) severe adverse consequences to lobular-alveolar development, and (2) positive effects on tumorigenesis or tumor progression leading to increased invasion and metastasis. To determine the role of p120 inactivation in breast cancer, we have proposed to study the consequences of targeted p120 loss of function in the mammary glands of normal and transgenic mouse models for tumorigenesis and metastasis. The first aim proposes to use gene targeting to incorporate loxP sites at strategic locations in the p120 gene such that Cre recombinase-induced deletion of the intervening sequence will inactivate p120. Mice containing this conditional allele ($p120^{flox}$) will be generated and crossed with WAP-Cre mice to target the conditional deletion to the breast. Our major progress in this reporting period is successful testing of the Cre-induced recombination event in the targeting construct, and the generation of 397 ES cell lines, some of which appear to be expressing the homologously recombined floxed p120 allele. Thus, we anticipate successful generation of the p120 knockout mouse in the next six months. The mouse will be invaluable for studying further the role of p120 in metastasis and breast cancer.

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INTRODUCTION

Accumulating evidence indicates that defects in the cytoplasmic mediators of cadherin function, the catenins, may account for cadherin dysfunction in carcinomas where E-cadherin expression is apparently normal. To determine the role of p120-catenin inactivation in breast cancer, we are studying the consequences of targeted p120 loss of function in the mammary glands of normal and transgenic mouse models for tumorigenesis and metastasis. We have used gene targeting to incorporate loxP sites at strategic locations in the p120 gene such that Cre recombinase-induced deletion of the intervening sequence will inactivate p120. Mice containing this conditional allele ($p120^{flox}$) will be generated and crossed with WAP-Cre mice to target the conditional deletion to the breast. We will determine the effects of selective p120 loss in the breast with respect to lobular-alveolar development, cadherin function, tumor progression, and metastasis. Our working hypothesis is that induced p120 loss in the breast will impair E-cadherin function leading to (1) severe adverse consequences to lobular-alveolar development, and (2) an acceleration of tumorigenesis or tumor progression leading to increased invasion and metastasis. Understanding the role of p120 in these processes may lead to new strategies for pharmacologic intervention as a means of inhibiting metatasis.

BODY

The statement of work for this grant is included below for reference. We have completed the targeting construct necessary to generate the conditional p120 knockout mouse. Our only modification of the original scheme was the insertion of frt motifs on either side of the neo cassette. In some instances, unscheduled splicing into the neo construct has been problematic for others. The modification makes it possible to remove neo from recombined p120 gene before moving the ES cells into mice. We have encountered delays of approximately six months as a result of this modification, but we believe that the overall chances of success are greatly increased. Currently, we have 397 ES clones that potentially have homologously recombined the construct into the mouse genome in place of the endogenous mouse gene. We are in the midst of a two part screening process that initially identifies the homologous recombinants, and subsequently isolates recombinants that have removed the frt cassette. Essentially, the most difficult tasks in the process of generating this mouse are nearly completed. An important development is that we successfully recombined the floxed p120 construct in vitro using soluble Cre. This is ultimately the reaction that needs to work in the mouse once the targeting construct is introduced into the mouse genome. Therefore, there is a high probability for success when the genetic work in the mouse is completed.

We have fallen behind by approximately six months, but should be ready to move forward with generating mice (Task 3) by January of 2002. Tasks 4 and 5 await the generation and characterization of the mice. Overall, the project is moving along as expected and there are no major problems to report. Recent developments in the p120/E-cadherin field indicate that this mouse will be invaluable for understanding the role of p120 in breast cancer metastasis, and we remain optimistic that the experiments will be successful.

STATEMENT OF WORK

Specific Aims 1 and 2: Role of the catenin p120 in Breast Cancer

Task 1: Months 1 - 6. Generation of the knockin contruct designed to introduce Cre-lox sites into the introns flanking the sequence to be deleted.

A. Insert the 4.639 kb genomic p120 sequence containing exons 3 - 5 into pBS246 and sequence critical regions to verify absence of mutations.

B. Subclone the targeting arms (regions flanking exons 3 - 5) into sites flanking the Not I site in pBS to generate pBS-A/C.

C. Insert the pBS246 Not I cassette into pBS-A/C to generate the final targeting vector.

Task 2: Months 3 - 9. Generation of floxed p120 ES cell lines

A. Transfect ES cells with the Cre-loxP targeting construct, select transfectants in G418, and screen for homologous recombination by southern analysis.

B. Generate the appropriate recombination event by transient transfection of Cre recombinase, gancyclovir selection, and analysis by PCR.

C. Test the cell lines in vitro to verify the ability to induce p120 loss in the presence of Cre

Task 3: Months 9 - 15. Generate floxed p120 mice
A. Blastocyst injections by the Vanderbilt Transgenic Core and generation of several founder lines with germline transmission containing the heterozygous floxed p120 allele.

Task 4: Months 15 - 24. Generate homozygous $p120^{flox}$ / WAP-Cre mice
A. $p120^{flox}$ / + and $tg^{WAP\text{-}Cre}$ / + crosses to generate experimental and control mice.
B. Characterization of the resulting mice by PCR and southern blotting.

Task 5: Months 24 - 36. Determine the effects of p120 loss in normal and abberant breast function.
A. Determine effects of p120 loss on lobular-alveolar development and function.
B. Test the long term effects of p120 loss in mammary tumor progression and malignancy by crossing the $p120^{flox}$/WAP-Cre mice with TAg and/or MMTV-MT mouse models for mammary carcinogenesis.

KEY RESEARCH ACCOMPLISHMENTS (bullet format)

*Inserted lox-P sites into introns flanking exons 1 – 7.
*Introduced frt sites on either side of the neo cassette.
*Ligated fragments of the above constructs to generate the targeting vector, containing floxed p120 sequences and a frt flanked neo cassette.
*Successfully tested the removal of the genomic sequence between the loxP sites in vitro.
*generated 397 ES clones containing the transfected targeting construct.
*completed the initial screening for homologous recombination.

REPORTABLE OUTCOMES

This is a long term project requiring generation of a conditional knockout mouse. Although the project is behind by six months, the successful test of the recombination reaction makes it unlikely that we will fail once the mouse is generated. Therefore, I remain optimistic, even though we are unlikely to have a reportable outcome until the mouse is available. We will not be able to complete all of the work by the end of the funding period. However, the mouse model will be completed, and the availability of the p120 conditional knockout mouse along with preliminary data derived from this model, will be an excellent starting point for NIH RO1 funding to explore further the role of p120 and the E-cadherin complex in breast cancer and metastasis.

CONCLUSIONS

As described above, we have completed a significant body of work resulting in the floxed p120 targeting vector necessary to generate the conditional p120 knockout mouse. Moreover, we have demonstrated successful removal of the floxed sequence by Cre, making it highly likely that the desired reaction be successful in the mouse. So far, we have made only minor modification to the original strategy and everything is proceeding as planned. We hope to have the mouse within six months, at which point we can begin to address the really interesting biological problems relevant to breast cancer metastasis.

REFERENCES: none
APPENDICES: none